Cytoskeleton: Axons Earn Their Stripes

Axons must be supported by a strong and flexible cytoskeleton. New 'super-resolution' imaging of the submembranous axonal cytoskeleton reveals that it is organized in a periodic, ladder-like structure with alternating rings of actin linked together by intervening complexes of spectrin.

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One hallmark of neurons is their complex cytoarchitecture involving distinct dendritic and axonal domains. Functionally, dendrites receive input from presynaptic partners, while axons propagate action potentials from the neuron to targets throughout the nervous system and body. Remarkably, the length of axons can be many thousands of times the diameter of the neuronal cell body; however, the structural mechanisms that support axon integrity over these immense distances are not well understood.

Neurons use a variety of cytoskeletal elements to maintain their morphologies, including microtubules, neurofilaments, and actin filaments. Whereas axonal microtubules and neurofilaments have been well-described using electron microscopy, the submembranous, actin-based axonal cytoskeleton has been little studied. In a recent paper in *Science*, Xu *et al.* [1] used stochastic optical reconstruction microscopy (STORM) — a new 'super-resolution' fluorescence imaging technique that permits resolution down to ~10 nm — to examine the detailed ultrastructure of the actin-based cytoskeleton in dendrites and axons. They found that in dendrites actin is organized in long filaments that run parallel to the long axis of the dendrite. Axonal actin had a very different distribution, however, and was organized in regularly spaced rings that wrapped around the axon just beneath the plasma membrane.

The submembranous cytoskeleton of axons is composed not only of actin, but also spectrins, ankyrins, and adducins [2]. Based on work in red blood cells, spectrins are thought to form hetero-tetramers consisting of two α- and two β-spectrins arranged in an anti-parallel fashion, with adducin-capped actin filaments found at each end of the tetramer. Ankyrins, on the other hand, bind near the carboxyl terminus of β-spectrins, or the middle of the spectrin tetramer, and function to anchor membrane proteins to the actin–spectrin cytoskeleton. By imaging these proteins using STORM, Xu *et al.* [1] revealed that they are also organized in a periodic network that colocalizes (adducin) or precisely alternates (spectrin and ankyrin) with actin (Figure 1A). Treatment of neurons with latrunculin A to depolymerize actin disrupted the regular spacing of spectrin, further supporting the notion that actin and spectrin form a highly organized and co-dependent network throughout the axon.

What benefits might this cytoskeletal organization confer on an axon? Axons are subjected to a variety of mechanical forces, including tension, compression, and torsion. Therefore, they must be flexible and strong and able to withstand the forces applied without snapping. Indeed, mutant worms lacking spectrins have axons that break under mechanical strain [3]. One of the simplest ways to dissipate these mechanical forces is to have a series of 'beams' and 'trusses' that create a latticework. Once a force is applied, it can be dissipated and spread throughout the series of compressible and extensible elements that comprise the latticework. It is attractive to speculate that the periodic structure of actin and spectrin functions in exactly this way [4].

Surprisingly, the alternating distribution of actin and spectrin was found to begin in the axon initial segment (AIS), adjacent to the cell body, and to extend throughout the distal axon. This is remarkable since the AIS and distal axon have different kinds of spectrins and ankyrins. For example, the distal axon is enriched with αII spectrin, βII spectrin, and ankyrinB (ankB) [5], while the AIS is highly enriched with βIV spectrin and ankyrinG (ankG). Thus, the unique distributions of axonal spectrins and ankyrins are unlikely to depend on actin. Apparently, even different kinds of axonal spectrins and ankyrins assemble in a similar periodic structure. One caveat to the idea that the spectrin tetramer defines the periodic spacing is that an α-spectrin has not yet been identified at the AIS. Furthermore, βIV spectrin is found in two different splice variants at the AIS, one with an amino-terminal actin-binding domain (βIVΣ1), and one without (βIVΣ6) [6]. The short form, βIVΣ6, is thought to be the major splice variant in mature neurons [7], and mice lacking βIVΣ1 still have ankG, sodium channels, and βIVΣ6 spectrin clustered at the AIS [8]. Thus, it is not clear how the regular periodic structure arises at the AIS without α-spectrin and with a β-spectrin isoform that lacks the actin-binding domain. It is possible that other, as yet unidentified proteins also contribute to the assembly of the periodic submembranous cytoskeleton in the AIS.

αII spectrin, βII spectrin, and ankB in the distal axon have been shown to function as an intra-axonal boundary to limit the position of ankG and βIV spectrin to the proximal axon [5]. However, in terms of the timing of events, Xu *et al.* [1] showed that the assembly of the periodic structure of actin arose after ankG clustering is normally seen in cultured neurons [5], suggesting that actin is unlikely to participate in establishing the intra-axonal boundary. AnkG and βIV spectrin cluster the ion channels necessary for action potential initiation [9], and maintain neuronal polarity by creating a diffusion barrier that restricts membrane proteins and trafficking vesicles to axonal or somatodendritic domains [10]. There is experimental support for the idea that the AIS barrier is actin dependent, since actin disruption permits the mixing of axonal and dendritic membrane proteins [11] and the entry of dendritic vesicles into axons [12]. One recent study even reported actin 'patches' within the AIS that function as vesicle filters [13]. However, the super-resolution images of Xu *et al.* [1] do not show these kinds of patches and suggest that actin is restricted mainly to the submembranous cytoskeleton. One potential explanation for the difference in these studies is that the detection of the actin patches required treatment of neurons with detergent before fixation, possibly resulting in the collapse

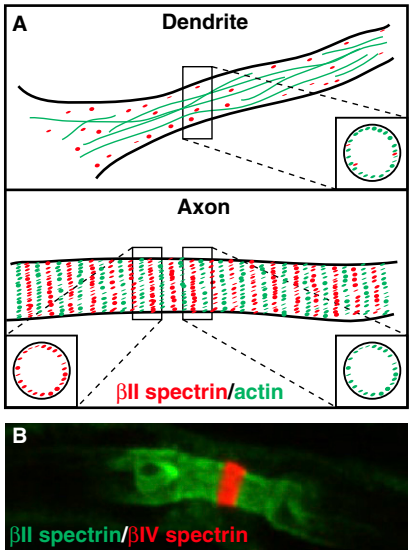

Figure 1. The organization of dendritic and axonal cytoskeletons.

(A) The submembranous actin–spectrin-based cytoskeleton is organized as a periodic, ladder-like structure. (B) Myelinated axons have high densities of different β-spectrins at and near nodes of Ranvier. (Image: courtesy of K. Susuki.)

of actin filaments into patches or aggregates. Thus, the experiments of Xu *et al.* [1] suggest that actin is not directly responsible for the AIS filter, but rather may participate in the stabilization or maintenance of some other macromolecular complex that functions as the AIS filter.

The axons examined by Xu *et al.* [1] were unmyelinated. However, a very large percentage of the axons in the brain and nervous system are myelinated. These myelinated axons do not have uniform cytoskeletons, but instead are divided into functionally and molecularly distinct domains, such as nodes of Ranvier and paranodal junctions, which, like the AIS, are highly enriched in spectrins and ankyrins. For example, nodes have clustered ankG and βIV spectrin, whereas paranodes, the sites flanking nodes where the myelin sheath attaches to the axon, are highly enriched in αII spectrin, βII spectrin, and ankB (Figure 1B) [14]. Cytoskeletal organization in myelinated axons depends on neuron–glia interactions. In future studies it will be interesting to determine how the internodal, paranodal, and nodal organization of the actin–spectrin cytoskeleton is modified by myelinating glia, or whether it resembles the organization of unmyelinated axons.

Finally, although all vertebrate cells have submembranous cytoskeletons composed of actin, spectrin, and ankyrins [2], the difference between the dendritic and axonal organization of actin and spectrin shows how the cytoskeleton may be organized differently in unique cellular compartments, even within the same cell. The methodologies used by Xu *et al.* [1] can be easily applied to many cell types to determine whether the periodic organization of the cytoskeleton is an invention unique to axons, or whether other cell types have arrived at similar or different solutions to the problem of how to maintain cellular architecture and integrity.

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Episodic Memory: A Rat Model of Source Memory

A recent study using a novel procedure to test the memory of rats for a preferred (chocolate) reinforcement shows many key characteristics that define source memory and episodic memory in humans.

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All animals have memory. But an important issue is whether episodic memory — that based on conscious recollection — is unique to humans or is shared by other (nonhuman) animals. This important issue is addressed in this issue of *Current Biology* by a paper from Crystal *et al.* [1]. First, some background on why episodic memory is an important memory type. There are two accepted major categories of long-term memory: declarative memory, that is explicit, conscious recollection; and nondeclarative or